

The diagnostic value of circulating microRNAs as a biomarker for gastric cancer: A meta-analysis

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Abstract. Recently, cancer research microRNA studies have drawn great attention. However, the results of these studies have been inconsistent and variable regarding the availability of circulating miRNAs in gastric cancer (GC) diagnosis. Thus, results should be interpreted cautiously. The purpose of the present study was to assess the diagnostic performance of circulating miRNAs in GC diagnosis. We conducted a systematic and comprehensive approach for the inclusion of studies. The sensitivity, specificity, and diagnostic odds ratio were pooled with random effects models, and a summary of receiver operator characteristic (SROC) curves were plotted. The potential heterogeneity was assessed with Q test and I² statistics. Subgroup analyses and meta-regressions further investigated the sources of heterogeneity. A total of 77 studies from 48 articles were eligible for the meta-analysis. The results revealed a sensitivity of 0.76, a specificity of 0.81, and an AUC of 0.86 for gastric cancer diagnosis with circulating miRNAs. In addition, subgroup analyses indicated that multiple miRNAs assays, non-microarray screening approaches, and serum-based miRNA assays exhibited good diagnostic performance in contrast to a single miRNA assay,

microarray expression profiling screening, and plasma-based miRNA group analysis. The diagnostic ability of miRNAs in early stage I-II groups and the high expression group were approximately similar to that in the stage I-IV groups and the low expression group. For the circulating miRNAs, our meta-analysis identified a combination of multiple miRNAs, non-microarray chip screening, and serum-based miRNA assays were associated with the most effective GC diagnostic performance. However, many unclear molecular mechanisms limited the accuracy of the diagnostic results, and should be interpreted with caution. Further large-scale prospective studies are required for validating the diagnostic applicability of circulating miRNAs in gastric cancer patients.

Introduction

Gastric cancer (GC) is the sixth most common cancer and the fourth leading cause of cancer-related deaths worldwide (1-3). The incidence of GC, especially in the Asian region (China, Japan, and Korea), has markedly increased over the past three decades (4). Moreover, statistics of cancer research from the International Agency for Research on Cancer have revealed that the morbidity and mortality rates associated with GC in China accounted for 42.6 and 45.0% of the global rates in 2015, respectively (5). Consequently, the high incidence of GC can result in great health and economic burdens (6). The recent development of diagnostic and surgical techniques has greatly improved the prognosis of GC patients (7,8). Five-year survival rates for patients with early GC can reach 90%. Unfortunately, most patients, however, have already developed advanced GC at the time of diagnosis (9,10). For patients with advanced GC, several studies have revealed that the median survival time of patients with GC was only 6-9 months, with surgical treatment unable to prolong the patient survival (11,12). Even with expanded resection, lymph node clearance, and neoadjuvant chemotherapy, the incidence rates of postoperative local recurrence and distant metastasis are still high (13). Therefore, considering the high ratio of early GC patients who are cured, the key to reducing mortality rates and improving the prognosis for GC patients is early and accurate diagnosis.

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Abbreviations: AUROC, area under the receiver operating characteristic curve; CI, confidence interval; DOR, diagnostic odds ratio; FN, false negative; FP, false positive; GC, gastric cancer; LR, likelihood ratio; QUADAS, quality assessment of diagnostic accuracy studies; SE, sensitivity; SP, specificity; TP, true positive; TN, true negative; TNM, tumor-node-metastasis

Key words: microRNAs, GC, circulation, meta-analysis, biomarker

To create a non-invasive, convenient, and low-priced diagnostic method, circulating biomarker detection methods have been widely used in clinics. GC serum tumor markers are currently used in clinical settings for the detection of GC. However, methods for the detection of carcinoembryonic antigen (CEA), carbohydrate antigen 199 (CA199), and carbohydrate antigen 724 (CA724) lack adequate sensitivity and specificity, which has precluded their widespread application in early diagnosis of GC (14). Therefore, it is necessary to investigate other potential biomarkers useful in identifying GC with high sensitivity and specificity. Recently, many researchers have been attracted to microRNAs (miRNAs) which have been stably detected in cell-free body fluids, such as plasma or serum. These miRNAs are called circulating miRNAs and are protected from degradation by ribonucleases in the blood. Additionally, the expression profile of miRNAs in GC patients usually exhibits exceptionally high expression of miRNAs in contrast to that in normal specimens (15).

miRNAs are a small, single-stranded non-coding RNAs that can regulate the expression of thousands of genes by inhibiting and degrading mRNAs during the translation process (16,17). miRNAs regulate various pathophysiological processes that involve cell proliferation, apoptosis, and differentiation. They also play an important role in tumorigenesis, especially neoplasm development, metastasis, angiogenesis, and immune responses (18).

Circulating miRNAs are considered to be novel potential biomarkers in the detection of many diseases, which were first demonstrated by Mitchell *et al.* (19) for the detection of cancer. Subsequently, their roles have been demonstrated in numerous studies. Nevertheless, it is difficult to specify a suitable miRNA, and the results of miRNA expression analysis are inconsistent. Despite a large number of studies on circulating miRNAs, few relevant measures have been applied in the clinical setting. In the present study, in this meta-analysis, we systematically and comprehensively analyzed the diagnostic accuracy of circulating miRNAs in distinguishing gastric cancer patients from the significant heterogeneity of other factors.

Materials and methods

Literature search. Two authors independently searched online databases, PubMed, Embase, the Cochrane Library, Web of Science, and Springer to identify potentially eligible studies published before November 10, 2017. The keywords used for literature retrieval were ('microRNA' or 'miRNA' or 'miR') and ('gastric cancer' or 'gastric carcinoma' or 'gastric tumor' or 'gastric neoplasm') and ('diagnostic' or 'diagnosis' or 'sensitivity and specificity' or 'ROC curve') and ('circulating' or 'plasma' or 'serum' or 'blood'). The citations in identified articles and in review articles were also examined. All publications identified by our search strategy were independently assessed by four reviewers. Any disagreement on a controversial study was resolved by discussion to consensus.

Literature selection. Eligible studies included in the present meta-analysis met the following criteria: i) the diagnosis of GC was made based on histopathological confirmation; ii) miRNA concentration in plasma, serum, or blood was detected before the patient received any treatment; iii) the study explored the

correlation between miRNA expression levels and gastric cancer diagnosis; iv) the study included standard references for the GC diagnosis, including patients with benign diseases or healthy individuals as the control groups; and v) the study provided adequate or sufficient data for the calculation of 2x2 tables consisting of true positive (TP), false positive (FP), true negative (TN), and false negative (FN). In addition, exclusion criteria were: i) publications irrelevant to the detection values of circulating miRNAs for GCs; ii) review studies, editorials, case reports, and letters; iii) duplicate publications; and iv) unqualified data. All literature that satisfied the above criteria constituted qualified studies.

Data extraction and quality assessment. The following data characteristics were extracted for each eligible study by two reviewers (HW and KP), independently: i) basic characteristics of the included studies, including the first author, year of publication, country of publication, origin of the study population, sample type, study design, sample size, number of participants, and variables adjusted for the analysis, and ii) for diagnostic studies, the sensitivity, specificity, TP, FP, FN, and TN values of the diagnostic test results were considered.

The quality assessment of the studies included in the present study was performed by two authors independently using the Quality Assessment of Diagnostic Accuracy studies (QUADAS-2) (20) checklist in Rev Man 5.0 software (<http://ims.cochrane.org/revman/download>). This scale was composed of four domains consisting of patient selection, index test, reference standard, and flow and timing domain. Each signaling question was judged as 'yes', 'no', or 'unclear' and each study's risk of bias and concern for applicability was estimated as 'high', 'low', or 'unclear' except for the flow and timing domain, for which the applicability concern did not apply. An answer of 'yes' meant that the risk of bias could be judged as being low, whereas an answer of 'no' or 'unclear' meant that the risk of bias could be judged as being high. A third reviewer was consulted in the case of conflict and inconsistency was dealt with by a multilateral argument.

Statistical methods. To evaluate the diagnostic accuracy of miRNA assays for detecting GC, the sensitivity and specificity parameters were extracted from each study and the number of patients with TP, FP, TN, and FN results from each included article were extracted or calculated. Based on the 2x2 tables, meta-analyses were performed with Meta-Disc software version 1.4 (http://www.hrc.es/investigacion/metadisc_en.htm) and STATA software version 12.0 (Stata Corporation, College Station, TX, USA) to evaluate the pooled statistics (95% CI) of sensitivity, specificity, positive and negative likelihood ratios (PLR and NLR) [PLR = sensitivity/(1 - specificity), NLR = (1 - sensitivity)/specificity], diagnostic odds ratio (DOR), and area under the summary receiver operating characteristic curves (AUSROC), with standard errors (SE) and Q index with SE for the test's performance using miRNA for GC detection. If sufficient information was not available, we recalculated these values on the basis of the sensitivity and specificity offered. Summary statistics revealed the diagnostic threshold effects that were analyzed by Spearman's correlation coefficient and P-value. If there was no significant threshold effect, the diagnostic accuracy was estimated by pooled

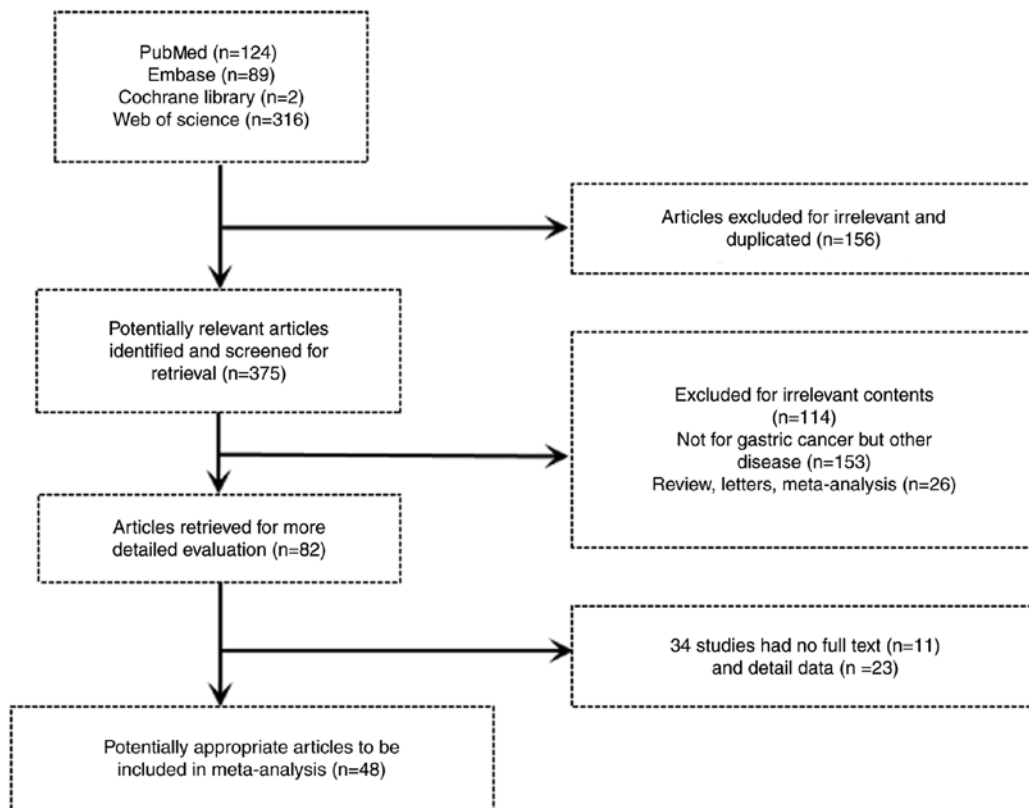


Figure 1. Flowchart for the selection of included articles is presented.

statistics, whereas the diagnostic accuracy was evaluated only by AUSROC and Q indices rather than sensitivities, specificities, PLR, NLR, and DOR.

Positive and negative likelihood ratios describe the discriminatory properties of positive and negative test results, respectively (21). Likelihood ratios state how many times more likely particular test results are in patients with disease than in those without disease (22). Positive likelihood ratios above 10 and negative likelihood ratios below 0.1 have been noted as providing convincing diagnostic evidence, whereas $PLR > 5.0$ and $NLR < 0.2$ imply higher diagnostic evidence (23). DOR represented the positive odds of aberrant miRNA expression in patients with GC compared to the probability of the healthy control. AUSROC values of 0.5-0.7, 0.7-0.9, and 0.9-1.0 were applied to indicate low, moderate, and high diagnostic accuracy, respectively. A smaller Q index indicated a lower diagnostic accuracy.

Heterogeneity was determined by Cochran's Q statistic based on the Chi-square test and I^2 statistics. I^2 values of 0-40, 40-70, and 70-100% indicated low, moderate, and high variance, respectively (24). If moderate heterogeneity existed or different clinical characteristics were noted, the DerSimonian and Laird random-effects model was applied. Considerable heterogeneity was considered if $I^2 > 50\%$ and/or $P < 0.05$ (25,26). Sources of heterogeneity were explored by meta-regression analysis based on possible characteristics and a subsequent subgroup analysis was performed to identify potential covariates. Furthermore, subgroup and meta-regression analyses were performed to detect the extent of heterogeneity between studies. Publication bias was checked using Deeks' funnel plot

analysis (24). All of the aforementioned statistical calculations were made with Meta-DiSc and STATA 12.0 software.

Results

Literature search. In Fig. 1, the flowchart for the selection of included articles is presented. Searching Pubmed, Embase, the Cochrane library, and Web of Science resulted in the inclusion of 531 articles. After a review of titles and abstracts, 156 publications were found to be irrelevant or duplicated. Next, we intensively read the remaining studies, whereby 114 of these publications were removed for irrelevant content, 153 articles were eliminated owing to the study being unrelated to gastric cancer, and 26 studies were not considered as they were review letters and meta-analyses. After a more detailed evaluation, 34 studies were removed as they did not contain full text or had insufficient data for extraction. Finally, the selection process revealed 48 studies that were eligible for diagnostic analysis.

Study characteristics and quality assessment. The main characteristics of the 48 qualified articles included 77 microRNAs, of which one study was performed in Europe and 47 studies were undertaken in Asia. The evaluated studies included a total of 3,829 cases and 3,175 controls for the present meta-analysis. These are presented in Table I. In the tumor-node-metastasis (TNM) classification, 20 articles included patients in stages I-IV. The other 13 included patients in stages I-II, of which only one study involved patients in stage I, whereas the remainder of the eligible studies (n=15) did not mention the TNM stage. Analysis of data from the nine included

Table I. Descriptive characteristics of the eligible studies.

Author	Year	Region	Case/control		Sample size (n)	Mean age (case/control) (year)	Male ratio (case/control) (%)	Sample/methods	miRNA profiling	Patients TNM (I/II/III/IV)	(Refs.)
			Case	Control							
Peng WZ	2014	China	57/58	NM	NM			Serum/qRT-PCR	miR-191	NM	(27)
Qiu X	2016	China	280/280	63.3 /63.2	63.2/63.2			Plasma/qRT-PCR	miR-26a	65/61/135/16/3 (I/II/III/IV/missing)	(28)
Tsujiura M	2015	Japan	104/65	NM	NM			Plasma/qRT-PCR	miR-18a	66/14/15/9	(29)
Zhang J	2015	China	155/111	56.6/47.4	71/63			Plasma/qRT-PCR	miR-16-5p, 19b-3p	33/26/59/25/12 (I/II/III/IV/unknown)	(30)
Tsai MM	2016	Taiwan	98/126	64.1/ 66.4	58.2/47.6			Plasma/qRT-PCR	miR-196a, 196b, and both	30/12/38/18	(31)
Su ZX	2014	China	82/65	69.0/71.2	58.5/60.0			Plasma/qRT-PCR	miR-18a	NM	(32)
Valladares-Ayerbes M	2012	Spain	52/15	65.9/65.3	81/80			Blood/qRT-PCR	miR-200c	9/12/31 (I-II/III/IV)	(33)
Zhu X	2017	China	114/95	NM	62.0/64.1			Serum/qRT-PCR	miR-145	35/79 (I+II/III+IV)	(34)
Zhuang K	2016	China	138/50	NM	61.6/NM			Plasma/qRT-PCR	miR-23b	NM	(35)
Hung PS	2017	Taiwan	65/108	67.7/56.1	72.3/62.0			Plasma/qRT-PCR	miR-376c	30/14/16/5	(36)
Huang S	2016	China	62/59	58/57	NM			Serum/qRT-PCR	miR-21, 31, 92a, 181b, 203	NM	(8)
Le Q	2017	China	41/41	NM	58.5/53.7			Blood/qRT-PCR	miR-25	NM	(37)
Li BS	2012	China	70/70	NM	70/63			Plasma/qRT-PCR	miR-223, 21, 218	12/11/36/11	(38)
Li F	2017	China	65/65	54.1/56.2	76.92/76.92			Plasma/RT-PCR	miR-106b, 93, 25	29/36 (I+II/III+IV)	(39)
Li M	2017	China	51/51	NM	62.7/NM			Plasma/qRT-PCR	miR-200c	NM	(40)
Hou X	2015	China	80/80	68.0/67.0	57.5/55			Plasma/qRT-PCR	miR-106a	45/35 (I+II/III+IV)	(41)
Hou CG	2016	China	150/150	NM	65.33/NM			Serum/qRT-PCR	miR-206	57/93 (I+II/III+IV)	(42)
Zhang WH	2012	China	20/20	60.9/NM	75/NM			Serum/qRT-PCR	miR-375	1/1/1/6/4/7 (Ib/II/IIIa/IIIb/IV/unknown)	(43)
Song MY	2012	China	68/68	NM	NM			Serum/qRT-PCR	miR-221, 376c, 744	NM	(44)
Tsujiura M	2010	Japan	69/30	NM	NM			Plasma/qRT-PCR	miR-106a/let-7a	38/13/14/4	(45)
Wang B	2012	China	30/39	58.0/46.0	73.3/23.1			Serum/qRT-PCR	miR-21	11/19 (I+II/III+IV)	(46)
Zhu C	2014	China	48/102	56.6/54.0	72.9/70.6			Plasma/qRT-PCR	miR-16, 25, 92a, 451, 486-5p, and combination	32/16(Ia/Ib)	(47)
Liu R	2011	China	82/ 64	60.2/60.0	84.0/78.0			Serum/qRT-PCR	Combined (miR-1, 20a, 27a, 34a, 423-5p)	29/56/48/23/8 (I/II/III/IV/unknown)	(48)
Konishi H	2012	Japan	56/30	NM	NM			Plasma/qRT-PCR	miR-451, 486	NM	(49)
Liu H	2012	China	40/41	56.0/58.0	65.6/65.6			Plasma/qRT-PCR	miR-378	4/12/11/13	(50)
Liu X	2016	Hong Kong	80/70	67.0/56.0	66.9/59.0			Plasma/qRT-PCR	miR-940	20/10/28/22	(51)
Hu Y	2016	China	137/79	NM	53.3/NM			Serum/qRT-PCR	miR-133a	58/79 (I+II/III+IV)	(52)

Table I. Continued.

Author	Year	Region	Case/control			Sample size (n)	Mean age (case/control) (year)	Male ratio (case/control) (%)	Sample/methods	miRNA profiling	Patients TNM (I/II/III/IV)	(Refs.)
			Mean age (year)	Male ratio (%)	Sample/methods							
Sun Y	2017	China	NM	96.7/61.5	76/26	NM	96.7/61.5	Serum/qRT-PCR	miR-183	15/10/30/21	(53)	
Li B	2017	China	NM	66.38/NM	116/85	NM	66.38/NM	Plasma/qRT-PCR	miR-320	68/48 (I+II/III+IV)	(54)	
Wu J	2015	China	NM	48.9/NM	90/90	NM	48.9/NM	Serum and PBMCs/qRT-PCR	miR-421	32/21/9/28	(55)	
Zeng Q	2014	China	NM	70/NM	40/36	NM	70/NM	Serum/qRT-PCR	miR-17, 106b and both	9/31 (I/II)/(III/IV)	(56)	
Zhou H	2012	China	NM	75/NM	40/17	NM	75/NM	Blood/qRT-PCR	miR-421	8/13/4/5/10	(57)	
Zhou X	2015	China	NM	65.6/61.1	32/18	NM	65.6/61.1	Plasma/qRT-PCR	Multiple	(I/II/III/IV/unknown)	(58)	
Wu J	2015	China	NM	48/NM	50/50	NM	48/NM	Serum and PBMCs/qRT-PCR	(miR-185, 20a, 210, 25, 92b) miR-21	9/11/10/18/2	(59)	
Wu D	2017	China	NM	NM	32/32	NM	NM	Serum samples/qRT-PCR	miR-503	NM	(60)	
Wang H	2014	China	NM	54/NM	50/47	NM	54/NM	Serum/qRT-PCR	miR-223, 16, 100	31/19 (I+II/III+IV)	(61)	
Shin VY	2015	Hong Kong	NM	NM	108/96	NM	NM	Plasma/qRT-PCR	Multiple	16/13/41/38	(62)	
Cui YJ	2015	China	NM	67.4/55	46/40	NM	67.4/55	Plasma/RFQ-PCR	(miR-627, 629, 652)	8/15/12/11	(63)	
Fu Z	2014	China	NM	47.36/NM	114/56	NM	47.36/NM	Plasma/qRT-PCR	miR-27b-3p	17/25/34/38	(64)	
Liu H	2017	China	54.3/53.6	62/64.1	137/145	54.3/53.6	62/64.1	Plasma/qRT-PCR	miR-217	43/94 (I+II/III+IV)	(65)	
Zhou H	2010	China	NM	NM	41/27	NM	NM	PMNCs/qRT-PCR	miR-106a, 17, both	NM	(66)	
Zhou X	2016	China	NM	NM	45/45	NM	NM	Serum/qRT-PCR	miR-223	NM	(67)	
Zheng Y	2011	China	NM	NM	52/20	NM	NM	Blood/qRT-PCR	miR-21	NM	(68)	
Li H	2017	China	NM	NM	75/38	NM	NM	Blood/qRT-PCR	miR-17-3p, 17-5p, 18a-5p, 19a-3p, 20a-5p, 92a-3p	NM	(69)	
Park JL	2015	Korea	51.8/48.9	51.4/51.4	35/35	51.8/48.9	51.4/51.4	Plasma/qRT-PCR	miR-27a	NM	(70)	
Li C	2013	China	56.7/58.9	68.8/64.3	80/70	56.7/58.9	68.8/64.3	Plasma/qRT-PCR	miR-199a-3p	69/7/4 (Ia/Ib/IIa)	(71)	
Li C	2013	China	58.1/58.9	68.9/65.4	180/130	58.1/58.9	68.9/65.4	Plasma/qRT-PCR	miR-199a-3p	40/29/93/18	(72)	
Liu S	2017	China	NM	NM	96/40	NM	NM	Serum/qRT-PCR	miR-144	47/49 (I+II/III+IV)	(73)	

TP, true positive; FP, false positive; FN, false negative; TN, true negative; SEN, sensitivity; SPE, specificity; AUSROC, area under the summary ROC curve; NM, not mentioned.

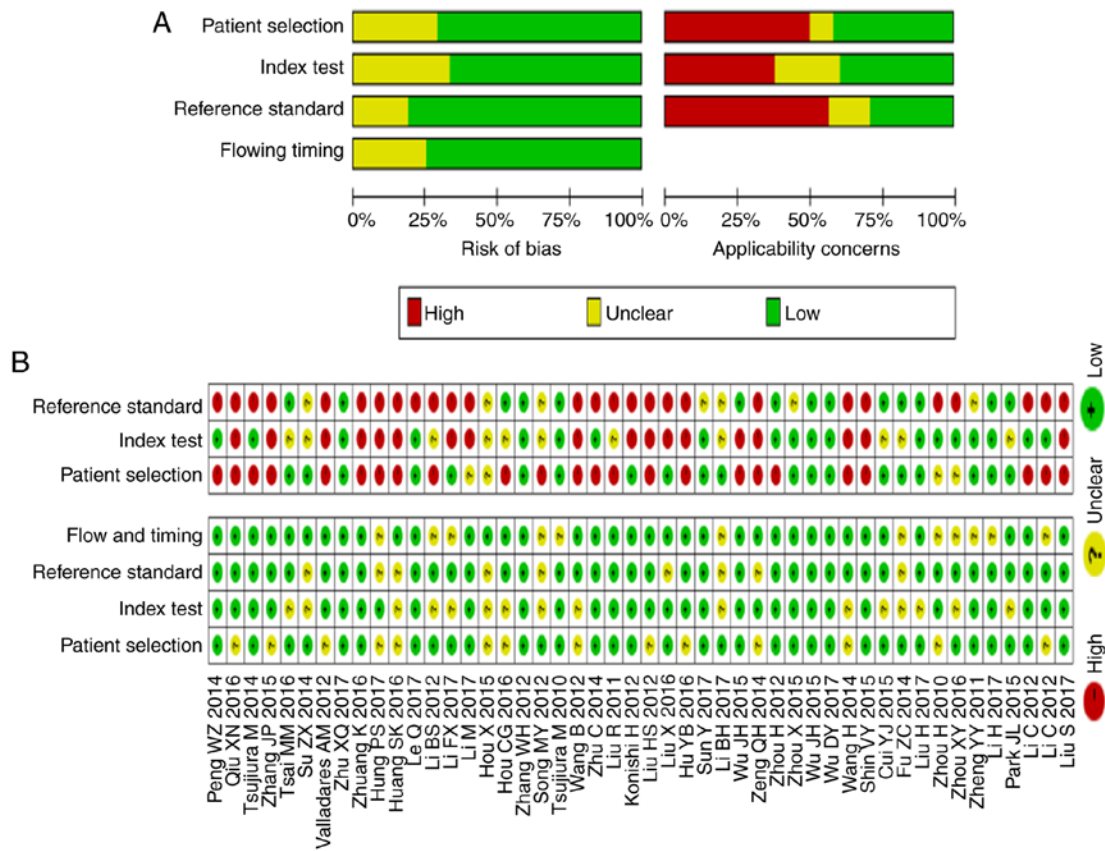


Figure 2. Assessment of the quality of the included studies using (A) a methodological quality graph and (B) the Cochrane Handbook.

studies that used miRNA microarray chips and revealed a number of miRNAs with altered expression, where candidate miRNAs were chosen via the training and validation design, whereas candidate miRNAs from the remaining articles (n=39) were chosen directly without microchip procession. Meanwhile, 42 of the 48 studies investigated the diagnostic value of a single miRNA used in GC detection, while only nine researched a set of miRNAs. Of the selected miRNAs, three were from the single miRNA studies. In terms of samples, circulating miRNAs from GC and healthy individuals were classified as serum (n=17), plasma (n=25), and peripheral blood (n=6). We also summarized miRNAs whose expression was upregulated in 33 studies and downregulated in 15 studies. One study (8) was excluded due to the unclear description about miRNA regulation, while another study (38) involved both upregulated and downregulated miRNAs. In particular, the expression of 58 miRNAs, including miR-21, was most frequently upregulated and that of 18 miRNAs (miR-26a and miR-199) was downregulated.

The quality of the eligible studies which were assessed, based on the QUADAS-2 criteria, was independently appraised by reviewers and is reported in Fig. 2A and B. In total, only five studies were valued as being low risk for bias and applicability concerns. The remaining studies were estimated as suboptimal for unclear risk in areas including index test, reference standard, flow, and timing. Most of the studies were identified as having a potential bias risk for patient selection and reference standard.

Diagnostic accuracy of circulating miRNA in GC. First, Meta-DiSc software version 1.4 was used to analyze the data.

The heterogeneity test found that Q test and I² values of DOR were 233.49 and 67.5%, respectively (P=0.0000) (Fig. 3A). Next, we generated forest plots of sensitivity and specificity, both of which did not display a straight line distribution and the Cochran-Q values were 369.52 and 555.63 (Fig. 3B and C), respectively, which reflected substantial heterogeneity (79.4 and 86.3%, respectively) among these studies. Random-effects models were then selected to re-analyze the data and the diagnostic threshold was analyzed. The Spearman's correlation coefficient was 0.253 (P=0.026), illustrating that the significant heterogeneity was partially caused by the diagnostic threshold. In addition, this may be caused by discrepancies in the study approaches, specimen type, endogenous reference, or total sample size. Thus, we could not calculate the statistical outcomes indirectly by neglecting the different factors and owing to the high heterogeneity. The data could not simply be pooled and was only suitable for subgroup analyses for illustrating heterogeneity.

Covariates and subgroup analysis. After stratification in accordance with the five pre-specified covariates, including patients' stage of GC (early TNM stage I-II vs. all TNM stages I-IV), miRNA profiling (single miRNA vs. multiple miRNAs), specimen types (plasma vs. serum vs. blood), miRNA screening approaches (microarray processing vs non-microarray processing), and aberrant expression (upregulation vs. down-regulation). we next assessed their impact on sensitivity or/and specificity as shown in Table II and Fig. 4. Comparing different TNM stages of GC patients with altered expression of circulating miRNAs, the results revealed that

Table II. Summary estimates of diagnostic criteria and their 95% confidence intervals.

Subgroup	N (miR)	SEN (95% CI)	SPE (95% CI)	PLR (95% CI)	NLR (95% CI)	DOR (95% CI)	I ² (%)	AUC (95% CI)
TNM stage								
TNM (I-II)	24	0.76 (0.72-0.79)	0.85 (0.81-0.89)	5.20 (4.16-6.52)	0.28 (0.25-0.32)	17.63 (13.82-22.48)	41.2	0.87 (0.84-0.89)
TNM (I-IV)	27	0.80 (0.75-0.84)	0.83 (0.76-0.88)	4.70 (3.32-6.66)	0.24 (0.20-0.29)	18.01 (12.56-25.81)	74.5	0.87 (0.84-0.90)
Unclassified TNM stage	26	0.76 (0.71-0.80)	0.83 (0.76-0.88)	4.40 (3.12-6.21)	0.29 (0.24-0.36)	12.18 (8.40-17.68)	67.5	0.85 (0.82-0.88)
miRNA screening								
Microarray	20	0.78 (0.72-0.83)	0.81 (0.72-0.87)	4.04 (2.81-5.80)	0.28 (0.22-0.35)	12.35 (8.63-17.68)	68.7	0.85 (0.82-0.88)
Unrelated microarray	57	0.77 (0.74-0.80)	0.85 (0.81-0.88)	5.01 (4.11-6.12)	0.27 (0.24-0.30)	17.20 (13.66-21.64)	66.7	0.87 (0.84-0.90)
miRNA expression profiling								
Single miR	68	0.77 (0.74-0.80)	0.84 (0.80-0.87)	4.69 (3.87-5.67)	0.28 (0.25-0.31)	17.00 (13.39-21.56)	67.2	0.86 (0.83-0.89)
Multiple miRs	9	0.80 (0.75-0.84)	0.85 (0.76-0.90)	5.21 (3.22-8.43)	0.23 (0.18-0.31)	22.22 (11.03-44.77)	68.2	0.87 (0.84-0.90)
Sample type								
Serum	23	0.81 (0.78-0.84)	0.83 (0.77-0.88)	4.89 (3.55-6.72)	0.23 (0.19-0.27)	19.44 (13.33-28.36)	65.8	0.88 (0.85-0.90)
Plasma	40	0.78 (0.74-0.81)	0.84 (0.79-0.88)	4.90 (3.74-6.43)	0.27 (0.23-0.31)	16.47 (12.62-21.49)	72.2	0.86 (0.83-0.89)
Peripheral blood	14	0.68 (0.61-0.74)	0.81 (0.75-0.86)	3.62 (2.81-4.65)	0.39 (0.33-0.47)	8.82 (6.36-12.24)	15.6	0.82 (0.79-0.85)
Altered miRNA ^a								
Upregulation	58	0.76 (0.73-0.79)	0.84 (0.80-0.87)	4.75 (3.84-5.88)	0.28 (0.25-0.32)	15.18 (11.98-19.25)	68.3	0.86 (0.82-0.89)
Downregulation	18	0.80 (0.75-0.84)	0.81 (0.75-0.82)	4.25 (3.22-5.61)	0.25 (0.20-0.31)	15.93 (11.61-21.86)	59.8	0.87 (0.84-0.90)

^aHuang *S et al* (8) was excluded due to the unclear description about miRNA regulation. SEN, sensitivity; SPE, specificity; NLR, negative likelihood ratio; PLR, positive likelihood ratio; DOR, diagnostic odds ratio; CI, confidence interval.

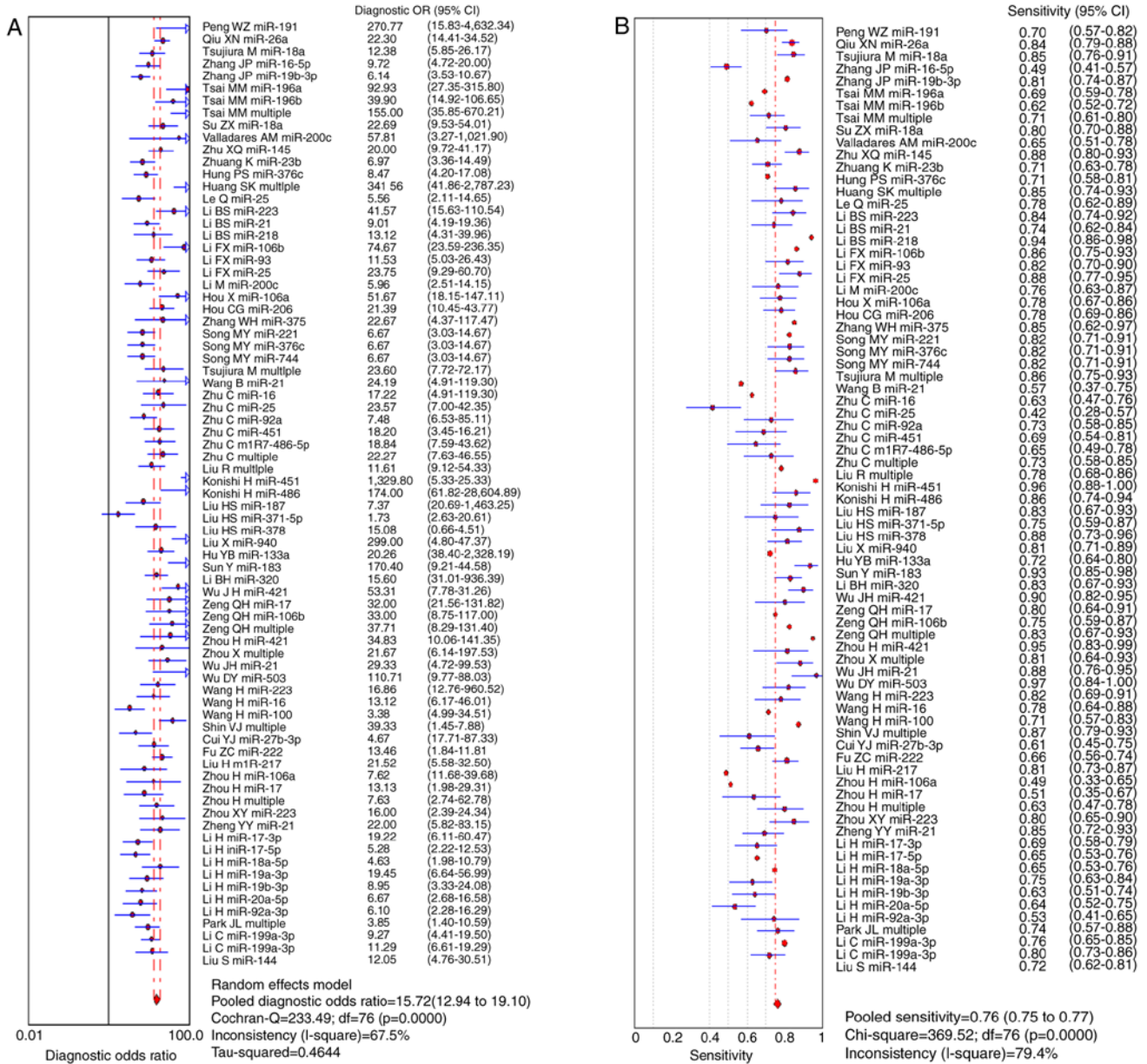


Figure 3. (A) Pooled diagnostic odds ratio (DOR) of circulating miRNAs in the diagnosis of gastric cancer patients. Forest plots and meta-analyses of studies showing the pooled (B) sensitivity of circulating miRNAs for diagnosing gastric cancer patients.

the diagnostic accuracy of miRNA detection during early stages I-II (sensitivity, 0.76; specificity, 0.85; PLR, 5.20; NLR, 0.28; DOR, 17.63; and AUC, 0.87) was similar to that during all stages I-IV (sensitivity, 0.80; specificity, 0.83; PLR, 4.70; NLR, 0.24; DOR, 18.01; and AUC, 0.87) and non-description stages (sensitivity, 0.76; specificity, 0.83; PLR, 4.40; NLR, 0.29; DOR, 12.18; and AUC, 0.85) with respect to all parameters except for the slight disparity in DOR. These results indicated that these biomarkers could not differentiate early GC from other later TNM stages in accordance with the diagnostic value.

Subsequently, we focused on the different screening approaches, such as microarray processing vs. non-microarray processing, and applied these approaches to candidate miRNAs. The differences between the pooled estimates of DOR (Table II) between miRNAs originating from microarray and miRNAs selected directly implied that the

diagnostic ability of the former was inferior to the latter. The AUSROC (Fig. 5) indicated that the diagnostic accuracy of miRNAs in microarray screening was slightly less than the non-microarray selection group. In addition, we conducted subgroup analyses based on miRNA profiling, including single miRNA and multiple miRNAs. In the subgroup analysis (Table II and Fig. 6), compared to that of single miRNA, the diagnostic ability of multiple miRNAs was better, with the sensitivity increasing from 0.77 to 0.80 and the specificity increasing from 0.84 to 0.85. AUC varied from 0.86 to 0.87 and the DOR value markedly increased from 17.00 to 22.22, whereas AUROC implied that there were parallel diagnostic accuracies between both.

Subgroup analysis of specimen types and aberrant expression were conducted to identify whether the candidate miRNAs could clearly discriminate GC sufficient diagnostic performance and accuracy. In the specimen type subgroup

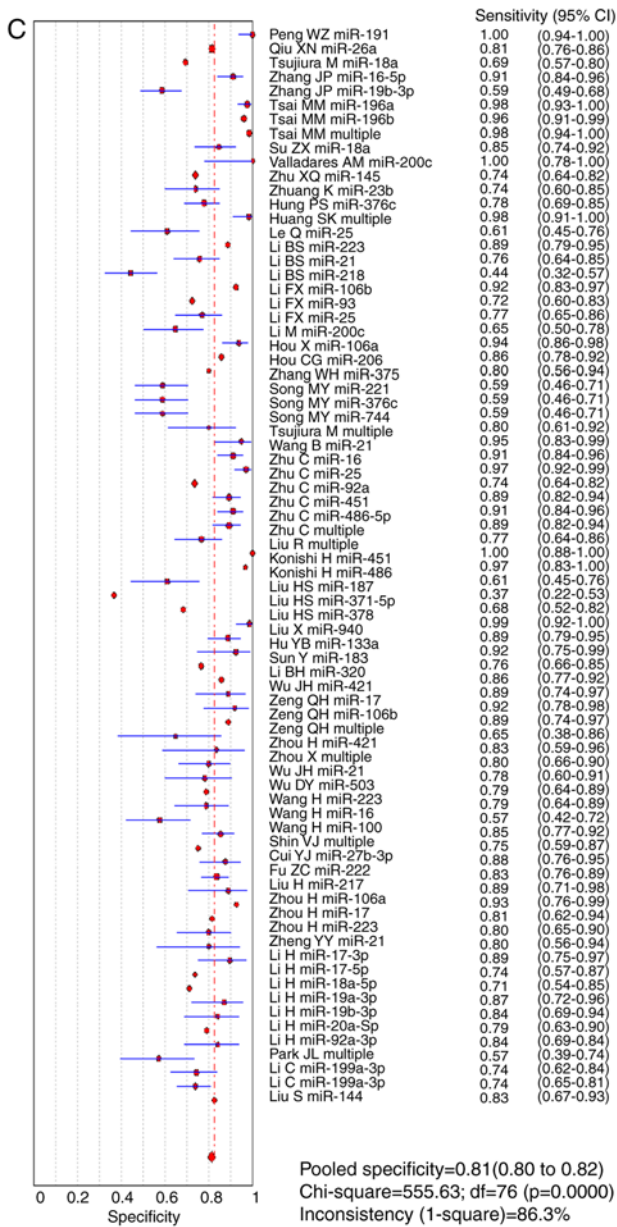


Figure 3. Continued. (C) Specificity of circulating miRNAs for diagnosing gastric cancer patients.

revealed in Table II and Fig. 7, the diagnostic sensitivity of miRNAs extracted from serum was 0.81 and the specificity was 0.83, with a pooled DOR of 19.44 and AUC of 0.88. The sensitivity and specificity of miRNAs from plasma-based studies were 0.78 and 0.84, respectively, with a summary DOR of 16.47 and AUC of 0.86. However, for the peripheral blood assay, sensitivity and specificity were 0.68 and 0.81, respectively, with a pooled DOR of 8.82 and AUC of 0.82, which indicated that the serum-based miRNA detection had a higher diagnostic value for GC than either the plasma or the peripheral blood-based assays. Moreover, further research was conducted to identify whether the upregulated miRNAs had better diagnostic accuracy than the downregulated miRNAs. Thus, the altered expression subgroup analyses for all miRNAs are shown in Table II and Fig. 8, the variable value in both changed slightly, according to the data of DOR (15.18 and 15.93) and AUC (0.86 and 0.87), which revealed that the diagnostic performance of miRNAs for GC detection in the high expression group was similar to that in the low expression group. From the above subgroup analysis that sought to determine the source of heterogeneity, the results indicated that the subgroups involving patients in the TNM (I-II) stage ($I^2=41.2\%$) using peripheral blood samples ($I^2=15.6\%$), contributed to moderate and mild heterogeneity, respectively. In addition, the decreasing trend in alteration implicitly suggested that the two factors may possibly be the source of heterogeneity. Nevertheless, further steps were taken to determine whether the aforementioned controversy led to the heterogeneity partly or entirely, which was confirmed by meta-regression analysis.

Meta-regression analysis. Based on the characteristics of the included studies, covariates, including TNM classification of GC, miRNA profiling, specimen types, miRNAs screening approaches, and aberrant expression status were applied to investigate inter-study heterogeneity using a meta-regression model shown in Table III (A-E). In the meta-regression analysis, sources of significant heterogeneity statistically indicated that the specimen type of the miRNA contributed significantly ($P=0.0014$), while the heterogeneity of results was not significantly influenced by the rest of the covariates. In accordance with the above

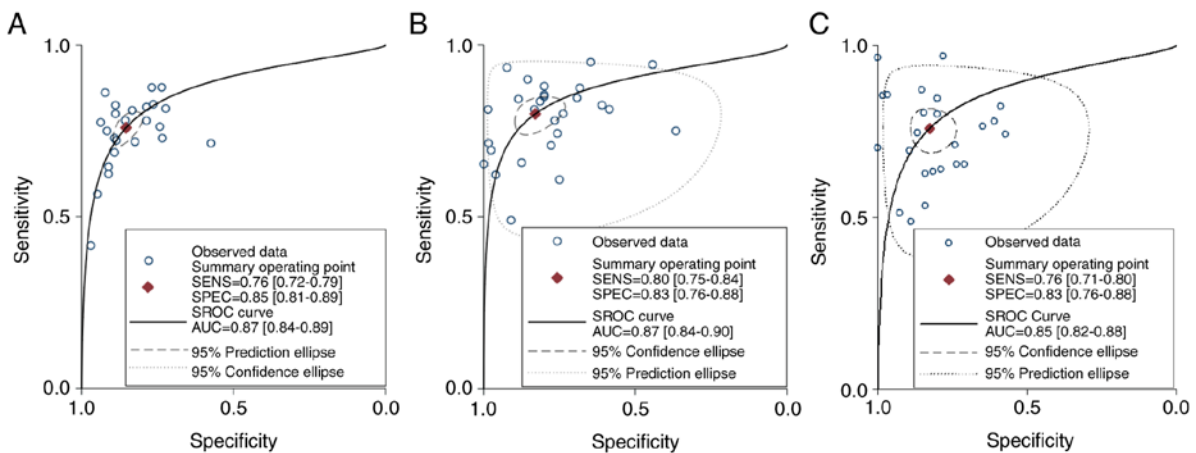


Figure 4. Summary of AUROC of circulating miRNAs from (A) early TNM stages (I-II), (B) TNM stages (I-IV), and (C) non-mentioned TNM stages for the diagnosis of gastric cancer patients.

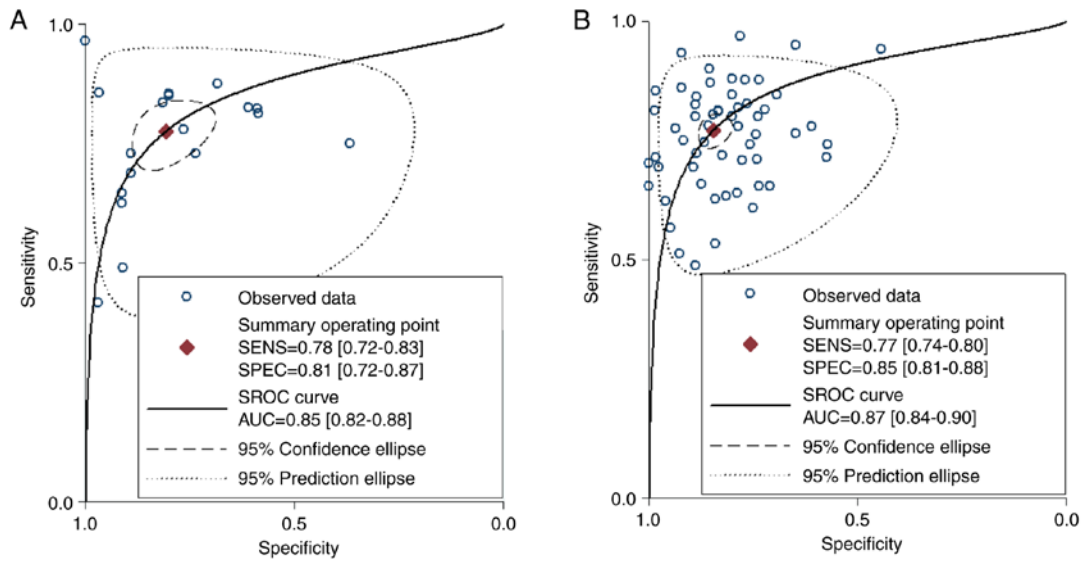


Figure 5. Summary of AUROC of circulating miRNAs from (A) microarray screening subgroup and (B) non-microarray screening subgroup for the diagnosis of gastric cancer patients.

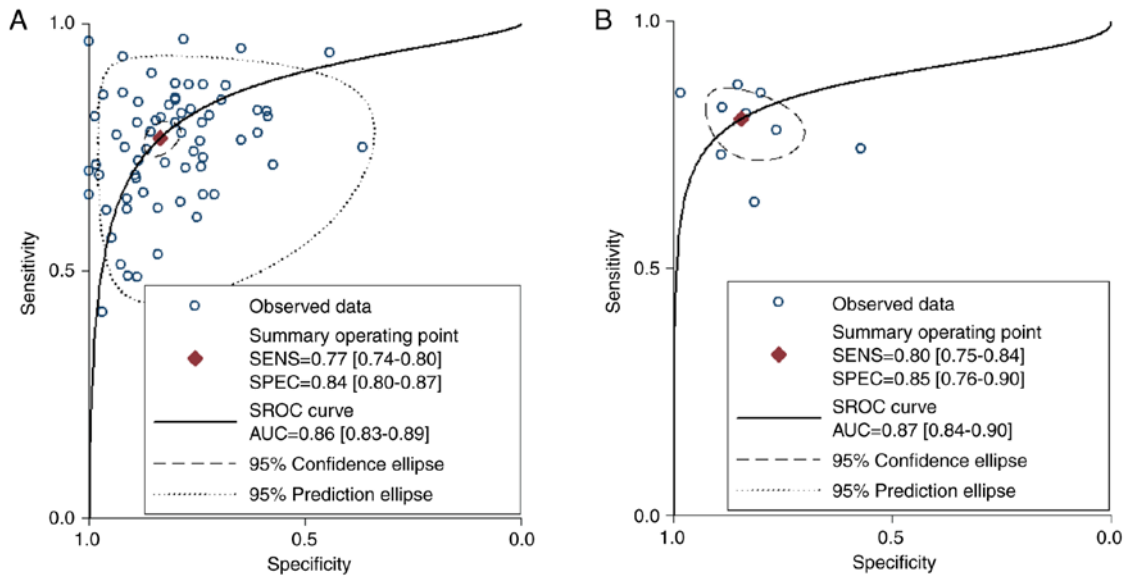


Figure 6. Summary of AUROC of circulating miRNAs from (A) a single miRNA and (B) multiple miRNAs for the diagnosis of gastric cancer patients.

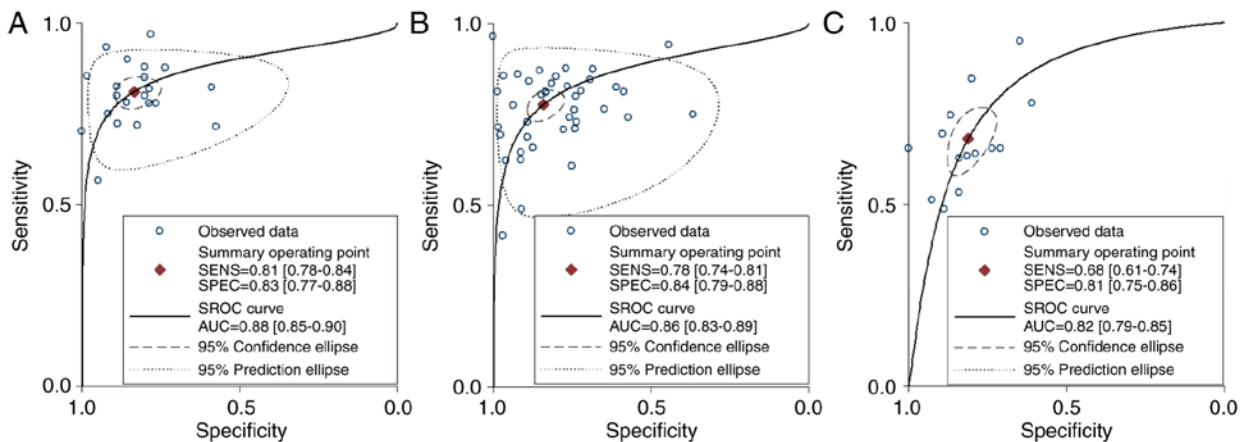


Figure 7. Summary of AUROC of circulating miRNAs from (A) serum-based specimens, (B) plasma-based specimens, and (C) peripheral blood-based specimens for the diagnosis of gastric cancer patients.

Table III. Meta regression to evaluate the inter-subgroup heterogeneity of miRNAs for the diagnosis of gastric cancer patients.

A, Five covariates					
Variables	Coeff.	Std. Err	P-value	RDOR	(95% CI)
Cte.	3.059	0.4343	0.0000	-	-
S	-0.301	0.0735	0.0001	-	-
TNM stage	0.186	0.1119	0.1004	1.20	(0.96;1.51)
Up/downregulation	-0.012	0.2047	0.9535	0.99	(0.66;1.49)
miR screening	-0.478	0.2051	0.0226	0.62	(0.41;0.93)
Sample type	-0.415	0.1361	0.0032	0.66	(0.50;0.87)
Single/multiple	0.254	0.2299	0.2728	1.29	(0.82;2.04)

Tau-squared estimate=0.2689 (convergence is achieved after 6 iterations). Restricted Maximum Likelihood estimation (REML). No. studies=48 containing 77 miRNAs. Filter OFF. Add 1/2 to all cells of the studies with zero. Cte, constant coefficient; S, statistic; RDOR, relative diagnostic odds ratio.

B, Four covariates					
Variables	Coeff.	Std. Err	P-value	RDOR	(95% CI)
Cte.	3.048	0.4183	0.0000	-	-
S	-0.300	0.0730	0.0001	-	-
TNM stage	0.188	0.1078	0.0851	1.21	(0.97;1.50)
miR screening	-0.479	0.2029	0.0209	0.62	(0.41;0.93)
Sample type	-0.416	0.1329	0.0025	0.66	(0.51;0.86)
Single/multiple	0.255	0.2272	0.2658	1.29	(0.82;2.03)

Tau-squared estimate=0.2606 (convergence is achieved after 6 iterations). Restricted Maximum Likelihood estimation (REML). No. studies=48 containing 77 miRNAs. Filter OFF. Add 1/2 to all cells of the studies with zero. Cte, constant coefficient; S, Statistic; RDOR, relative diagnostic odds ratio.

C, Three covariates					
Variables	Coeff.	Std. Err	P-value	RDOR	(95% CI)
Cte.	3.364	0.3156	0.0000	-	-
S	-0.328	0.0696	0.0000	-	-
TNM stage	0.176	0.1086	0.1085	1.19	(0.96;1.48)
miR screening	-0.402	0.1932	0.0409	0.67	(0.46;0.98)
Sample type	-0.428	0.1340	0.0021	0.65	(0.50;0.85)

Tau-squared estimate=0.2727 (convergence is achieved after 6 iterations). Restricted Maximum Likelihood estimation (REML). No. studies=48 containing 77 miRNAs. Filter OFF. Add 1/2 to all cells of the studies with zero. Cte, constant coefficient; S, Statistic; RDOR, relative diagnostic odds ratio.

D, Two covariates					
Variables	Coeff.	Std. Err	P-value	RDOR	(95% CI)
Cte.	3.630	0.2759	0.0000	-	-
S	-0.328	0.0706	0.0000	-	-
miR screening	-0.355	0.1944	0.0716	0.70	(0.48;1.03)
Sample type	-0.476	0.1328	0.0006	0.62	(0.48;0.81)

Tau-squared estimate=0.2899 (convergence is achieved after 6 iterations). Restricted Maximum Likelihood estimation (REML). No. studies=48 containing 77 miRNAs. Filter OFF. Add 1/2 to all cells of the studies with zero. Cte, constant coefficient; S, Statistic; RDOR, relative diagnostic odds ratio.

Table III. Continued.

E, One covariate					
Variables	Coeff.	Std. Err	P-value	RDOR	(95% CI)
Cte.	3.474	0.2661	0.0000	-	-
S	-0.335	0.0717	0.0000	-	-
Sample type	-0.445	0.1340	0.0014	0.64	(0.49;0.84)

Tau-squared estimate=0.3116 (convergence is achieved after 6 iterations). Restricted Maximum Likelihood estimation (REML). No. studies=48 containing 77 miRNAs. Filter OFF. Add 1/2 to all cells of the studies with zero. Cte, constant coefficient; S, Statistic; RDOR, relative diagnostic odds ratio.

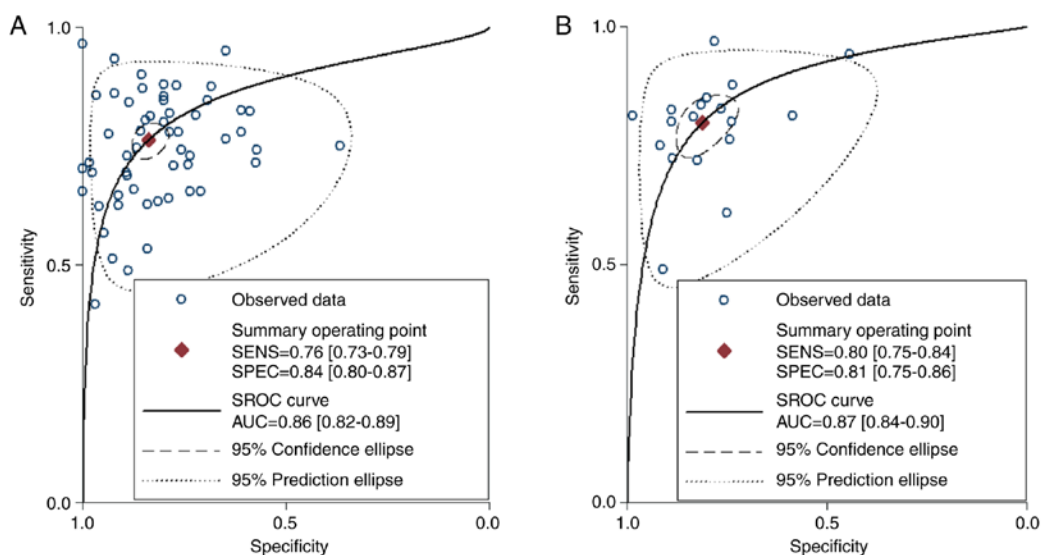


Figure 8. Summary of AUROC of circulating miRNAs from (A) upregulated miRNAs and (B) downregulated miRNAs for the diagnosis of gastric cancer patients.

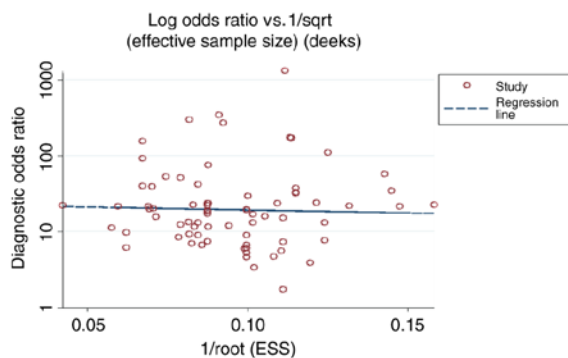


Figure 9. Deek's funnel plots used to estimate publication bias for discrimination of miRNAs in patients with GC. No evidence of publication bias was explored.

results, the study sample type could be considered as a source of heterogeneity for GC detection in meta-regression.

Publication bias. Deeks' funnel plot asymmetry test was applied to explore the publication bias of meta-analysis in diagnostic accuracy (24). The slope coefficient was associated with a P-value of 0.756 for GC detection (Fig. 9), suggesting a low likelihood of publication bias in our meta-analysis.

Discussion

GC is responsible for the highest number of cancer-related mortalities (74), and the egregious mortality of GC is immeasurably more acute than ever before, primarily since the majority of patients have a terminal disease at stage III or IV at the time of diagnosis (75). In addition, there are many investigated biomarkers, such as CEA and CA724, which lack sufficient sensitivity and specificity for early GC diagnosis (76), and universal screening tools, such as endoscopic examinations and biopsies, are invasive, unpleasant, and inconvenient, leading to potential errors in GC detection. Hence, ideal non-invasive biomarkers are urgently required to reinforce GC detection. At present, a large number of studies on the search for novel tumor biomarkers have revealed that miRNAs may play a pivotal role in cancer suppression, owing to the diverse miRNA expression levels that are observed between cancer patients and healthy controls (45,48,77,78). Subsequently, research has gravitated towards miRNAs as biological markers for tumor diagnosis. Nevertheless, the results of research on the use of miRNAs for gastrointestinal cancer detection are conflicting among different studies (33,38,44,45,47,48,79,80). To the best of our knowledge, several meta-analysis studies have been

undertaken to determine the differentially expressed miRNAs in GC patients. Unfortunately, as a result of insufficient data or inconsistent results, abundant heterogeneity influenced the results. Furthermore, the accuracy of performance using the pooled results influenced interpretation. In the present meta-analysis, we included 48 studies involving miRNA expression profiling to systematically and comprehensively evaluate the potential diagnostic value of circulating miRNAs as diagnostic markers for GC. We considered different perspectives while avoiding statistical outcomes that included the absence of homogeneity.

The pooled outcomes of sensitivity, specificity, and AUC (0.76, 0.81, and 0.86, respectively) with the random effects model revealed that circulating miRNAs have better diagnostic value than CEA and CAA199 (AUC of 0.55 and 0.60, respectively) in distinguishing GC patients from control groups. Moreover, the DOR of circulating miRNAs for GC detection was 15.72, reflecting higher diagnostic performance as a combinative parameter of sensitivity and specificity. In fact, by pooling data in this manner, the diagnostic value would be inaccurate due to the significant heterogeneity and diagnostic threshold. Thus, we could not interpret the statistical outcomes blindly while neglecting high heterogeneity. Additionally, it was suitable to explore subgroup and regression analyses.

From the subgroup analysis, our results indicated that the non-microarray screening approach, multiple miRNA assay, and serum-based miRNA assay manifested a relatively higher diagnostic value and accuracy for GC than the single-miRNA, microarray profiling screening, and plasma-based miRNA groups. The altered expression of the single miRNA in serum or plasma fluctuated not only in GC but also in other tumors, infectious diseases, nonspecific inflammation, and acute injuries. In other words, single miRNAs lacked specificity in cancer detection. However, for multiple miRNAs with complex molecular mechanisms, such as a competing endogenous RNA (ceRNA) network intersecting at tumorigenesis (e.g., initiation and development of a severe neoplasm), the association may be valuable for early GC detection. Hence, studies highlighting individual cancer-specific miRNAs in serum or plasma usually arrived at unsatisfactory results.

Non-microarray screening and serum-based diagnostic tests yielded better outcomes than microarray screening pathways and plasma-based investigations of GC. However, the origin of source-related differences was still unclear. There are many complex factors that must be accounted for, such as lower homogeneity of included studies and a limited number of samples. Therefore, large-scale investigations and multiple center trials should be undertaken in the future to uncover the underlying mechanism of aberrant expression of miRNAs and to determine whether the source-related discrepancies truly exist or not.

Another finding was that the diagnostic value of miRNAs in early stages I-II and high expression groups were approximately similar to those in stages I-IV and lower expression groups in the detection of GC. In the GC microenvironment, a variety of differentiated tumor cells and cancer-associated cells, such as different types of immune cells and cells with different proliferative activity, lead to the transcriptome dysfunction during the tumorigenesis process due to inactivation of tumor suppressors and activation of proto-oncogenes.

With respect to cancer, immune cells have the capacity to release exosomes that accompany cell migration, shuttling the ceRNA network into circulation (81), whereas the circulating tumor cells may express non-coding RNA under the control of an oncogene. Moreover, the overexpression of miRNAs may silence the mRNAs from tumor suppressor genes and down-regulated miRNAs may facilitate the expression of oncogene mRNAs by binding the 3'-untranslated region of the target mRNA. In early and advanced GC, the aberrant and abundant expression of some miRNAs may be associated with this process. Moreover, a lack of specificity in the aforementioned may occur not only in GC but in many tumors, leading to various conclusions regarding GC discrimination. For instance, Liu *et al* (50) suggested that miRNA expression levels during earlier stages (I and II) were different from those during later stages (III and IV), exhibiting that this miRNA could be valuable for the early detection of GC. Evidently, we arrived at a paradox with our results conflicting with the conclusions of these authors. Therefore, it is difficult to distinguish which is a suitable and specific biomarker for GC. It would be beneficial to study individual miRNAs by determining their molecular mechanism rather than using the combination methodology.

The present study does have some limitations that must be addressed. First, methodologies for a precise uniform quantification of miRNAs face a lack of consistent criteria, limiting the comparisons made between studies that are conducted by different laboratories who have their own study design, use of miRNA chips, pathology type, localization of GC lesions, and different endogenous miRNA references. Second, there are some specific circulating miRNAs that are always prone to be selected by certain studies in determining the correlation between grade and stage of cancer. Consequently, a standardized protocol, which would be preferable, is required to abate bias. Moreover, the included studies in the present meta-analysis only distinguished the tumor patients from healthy controls, but other risk factors, such as chronic gastritis, infectious disease, genetic, ulcers, and diet, were not included and these may contribute to altered miRNA expression (4,82). Therefore, further accurate studies on the use of miRNAs for distinguishing cancer from other diseases are urgently needed.

In conclusion, our meta-analysis found that the combination of multiple miRNAs, non-microarray chip screening, and serum-based miRNA assays may present a better performance for the diagnosis of GC. However, many unclear molecular mechanisms hindered discovery for clear GC detection biomarkers. Therefore, the results should be interpreted cautiously given the uncertainty of the results. Further large-scale prospective studies are required to validate the potential applicability in human cancer diagnosis.

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Availability of data and materials

All data generated or analyzed in this study are included in this published article.

Authors' contributions

HuiW, KP, XGL, BXL, HSZ, HuanW, HaoW, WMS and YPW contributed to the conception and design of the study, literature review and analysis, drafting and critical revision and editing, and approval of the final version.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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